##### Determination of Some Vitamins in Primary Brain Tumors Patients

Abdul-Wahab R. Hamad1, Khaled, N. Al-Kubaisy1, Walid W. Al-Rawi3, Raad K. Muslih4 and Nuha Auwaed Mashaly4.

1Department of Medical Allied of Sciences, Zarqa University College, Al-Balqa, Applied University, Jordan.

3College of Medicine, University of Duhok, Iraq.

4Department of Chemistry, Al-Mustansiriyah University, Iraq.

# Abstract: The requirements and / or the availability of various nutritional factors for the living cell(s) can be quite variable under both physiological and pathological situations. Vitamins are an established component of the spectrum of necessary constituents of food to the living tissues and many of them have proved a protective role in cancer aetiology. This is a prospective study to investigate the levels of few vitamins in sera, saliva, cerebrospinal fluid (CSF), and tumour tissues in the context of primary brain tumours (PBT), both benign and malignant, among a group of Iraqi patients. This study had been conducted between November 2011 and October 2012 at the Neurosurgical Hospitals. Out of the 107 patients suffering form PBT with an age range 2-75 years (mean 35, the SD ± 19), 56 were males (52.3%), and 51 were females (47.6%). The most affected age group was 31-40 years (17.75%), 89% of the patients were under the age of 60 years. There were 44 gliomas (both benign and anaplastic) and 32 meningiomas (benign). A group of 50 patients with congenital hydrocephalus were involved in the study for CSF sampling. Forty age- and sex-matched normal subjects were used as controls in serum and saliva measurements. High performance liquid chromatography (HPLC) was used in the study. All 3 vitamins have shown lower values in malignant tumours patients (in serum, saliva, CSF, and tumour tissues) compared to those with benign tumours and controls in the biological fluids and tissues that were assayed with statistical significance. These changes are quite significant, the findings should be considered in further research to determine both the specificity and sensitivity of low vitamins levels in the possible aetiological association with PBT.

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**Key words:** Primary brain tumour; vitamins A, C, and E; saliva, cerebrospinal fluid.

**Introduction**

Cancer is a major health problem, not just in industrialized nations, but also in developing nations around the world, that cannot be ignored. It is like other chronic disease not only caused by a single factor, but is by multifactorial events (like genetic, environmental factors, chemical agents, radiation, reactive oxygen species (WHO, 2003).

A brain tumor is a mass of abnormal cells that is growing in or around the brain. They are generally named after the type of cell they developed from, benign and malignant terms are used to describe these tumors (Thomas and Graham, 1980). Free radicals are continuously produced during aerobic metabolism. These unstable species may cause oxidative damage to DNA, carbohydrates, proteins and lipids that are normally counteracted by protective antioxidants (Bishop et al., 2005). Oxidative defense is provided by a number of enzymes and vitamins, including the chain-breaking scavengers vitamin E, vitamin C and glutathione (Halliwell, 1997). In times of increased free radical production, individuals may become deficient in these antioxidants.

Primary brain tumors are tumors that start in the brain. There are many types and subtypes of primary brain tumors; some are benign, others malignant. Examples include gliomas, meningomas, medullablastomas, pituitary adenomas, and central nervoussystemlymphomas.
Vitamins have proved and remained essential dietary components of the balanced diet that is needed for a healthy growth of the living organism. They have varieties of mechanisms of actions in the living tissues. Vitamin deficiency syndromes are well established and evidence-based clinical entities. Vitamins are needed in many physiological and pathological situations to meet the requirements of the living cells. Moreover, there are certain situations where the daily requirements become more than during ordinary times, such as that occurs during pregnancy. The figures that represent the daily allowances for the vitamin intake have been well established in the literature.

Classical and new therapies in anaplastic astrocytomas and glioblastomas do not yield sufficient results. Agents able to redifferentiate neoplastic cells in vitro are known. Patients with glioblastomas and anaplastic astrocytomas were enrolled in a phase II trial involving surgery or biopsy, radiotherapy (64 Gy), chemotherapy. Alfa calcidol, a vitamin D analog able to bind to nuclear receptors regulating mitotic activity, has been used in the treatment of malignant gliomas as an in vitro agent of redifferentiation; it is safe and seems able to induce in some patients, in synergy with classical surgery-radiotherapy-chemotherapy treatments, a particular progressive and durable regression of the tumor. The responders might represent about 20% of malignant gliomas (Trouillas et al. 2001).

A protective effect among glioma pairs relating to frequency of use of vitamin C and other vitamin supplementsPreston Martin et al. 1989).

Similarly, studies have been demonstrated that vitamin C (ascorbic acid) exhibit the protective role of vin in certain types of cancer (rat glial tumor cells); Vitamin C inhibited DNA adduct formation and arylamine N-acetyltransferase activity and gene expression in rat glial tumor cells (Hung and Lu, 2001).

The aim of the study is to investigate the levels of few vitamins in the context of PBT among a group of Iraqi patients (107 persons), in their sera, saliva, brain tumour tissues, and CSF and to compare the CSF figures with that of a cohort of 50 hydrocephalic patients and sera of another cohort of 40 healthy volunteers.

**Materials and Methods**

**Patients and methods**

This study had been conducted between November 2011 and October 2012 at the Neurosurgical Hospitals. Patients were evaluated by full medical history to exclude any existing systemic disease that may affect the parameters to be diagnosed, particularly diabetes, liver disease, renal disease and chronic drug intake, other wise the patient was excluded from the study.

Out of the 107 patients suffering form primary brain tumors with an age range 2-75 years (mean 35, the SD ± 19), 56 were males (52.3%), and 51 were females (47.6%). The most affected age group was 31-40 years (17.75%), 89% of the patients were under the age of 60 years (Table 1).

Age and sex distribution in 107 primary brain tumors patients is shown in Table 1.

There were 44 gliomas (both benign and anaplastic) and 32 meningiomas (benign ) .

Pathological grading showed that the highest percentage of the patients were of grade IV (34%) followed by grade III (27%)**.**

A group of 50 patients with congenital hydrocephalus were involved in the study for CSF sampling. Forty age- and sex-matched normal subjects were used as controls in serum and saliva measurements.

**Duration of the disease**

The duration of the disease range from <1->9 years. The majority of the patients (57.94%) presented within less than 1-year from onset of symptoms.

**Chemical and Reagents**

All Chemical and standard solutions used in this work, were the highest analytical grade obtained from commercial source, and used without further purification. All volumetric glassware were cleaned in a solution of 5 n HCL for at least 24 hrs, then washed repeatedly in deionzed water prior to use. Vitamins (A and E) standard were obtained from Supelco Park, Bellefont USA.

**Sample collection and preparation**

**Serum**

A 5 mlliliters. venous blood was drawn aseptically into sterile test tube with silicon coated, by utilizing disposable needle and plastic syringes. The blood was allowed to clot (10 minutes), centrifuged at 4000 rpm for 15 min. Serum sample were immediately transferred into four tube and frozen at (-20˚C) for subsequent analysis, haemolyzed samples were discarded.

# Table 1. Distribution of PBT patients according to age and sex

|  |  |  |  |
| --- | --- | --- | --- |
| **Age (years)** | **Male** | **Female** | **Total** |
| **1-10** | 5 (41.66%) | 7 (58.33%) | 12 (11.21%) |
| **11-20** | 10 (58.82%) | 7 (41.17%) | 17 (15.88%) |
| **21-30** | 9 (60%) | 6 (40%) | 15 (14.01%) |
| **31-40** | 10 (52.63%) | 9 (47.36%) | 19 (17.75%) |
| **41-50** | 8 (44.44%) | 10 (55.55%) | 18 (16.82%) |
| **51-60** | 8 (57.14%) | 6 (42.85%) | 14 (13.08%) |
| **61-70** | 4 (44.44%) | 5 (55.55%) | 9 (8.41%) |
| **>70** | 2 (66.66%) | 1 (33.33%) | 3 (2.80 %) |
| **Total** | 56 (52.33%) | 51 (47.66%) | 107 (100%) |

One milliliter of venous blood, after clothing, was centrifuged at 600 rpm for 10 min. serum was diluted with 0.3 ml (0.2 M) sodium potassium phosphate buffer (pH 8.4) and centrifuged for 20 min. at 1000 rpm thoroughly to remove protein. The filtrate was kept frozen at (-20˚C) until analyzed. (20-100 ml) aliquot of the filtrate was used for HPLC analysis. Assays were done at the laboratories of college of sciences, Jordan.

**Saliva**

Unstimulated whole saliva was collected after the patient have rinsed his mouth several timed with deionzed water, then the accumulated saliva in the floor of the mouth was drawn by a plastic disposable pipette, collection time was always between 8-9 a.m.

The collected saliva was centrifuged at 2500 rpm for 10 minutes at 5˚C, this was done within one hour after collection to eliminate debris and cellular matter. The centrifuged supernatants were divided into 5 equal parts. All sample were stored frozen at (-20˚C) in polyethylene tubes till assayed.

**Tumor tissue**

Tumor tissue was taken from the lesion at the day of surgery, which immediately transferred, for mincing and homogenization. An equal volume of Triton X-100 buffer (sodium – phosphate buffer) is added to the minced tissue, and then cold centrifugation was performed at 4000 rpm for 30 minutes at 4 ˚C. The centrifugal supernatant was aspirated and divided into 5 equal parts. All samples were stored frozen at (-20˚C) in polyethylene tube until assayed.

**CSF**

The 107 patient included in this study had fasted for 8 to 12 hrs., before surgery. A CSF specimen (3 to 4 ml) was collected in a plastic specimen container from each patient at the time of operation through a ventricular catheter.

CSF samples were collected via a ventricular catheter that was used in treatment. The CSF specimens were collected in plastic containers, promptly frozen, and stored at (-0˚C) until analysis. Assays were done within one week to one month of collection at the laboratories of The Neurosurgery Hospital.

**Vitamins (A, E and C)**

Chromatographic conditions used were established by Abid et al.,2002. The HPLC system used was LC-6A liquid chromatographic, equipped with UV-visible detector model SPD-6AV operating at 210 nm for water soluble vitamin (Vit.C) and 295 nm for fat soluble vitamins (A&E).

A Rheodyne 7125 valve injector with 100 Ml injection loop was used. SCL-6A system controller controlled the solvent delivery system. The resulted retention time and peak area were display and processed on chromatopack C-18 ODS (250 × 4.6 mm I.d.), 5Mm partied size, and propylamine column (250 × 4.6 mm I.d.),5Mm particle size, were used throughout this work. The column temperature was maintained at 40 oC using column’s oven model CTO-6A. Acetonitrile, methanol, sodium octane sulfonate, hexane and ethyl acetate were used.

The buffer sodium dihydrogen phosphate were prepared in deionized water and adjusted to pH 2.1 with sodium hydroxide. Flashing the eluent with stream of helium for 10 minutes degassed all eluent.

**Preparation of standards**

Vitamin C (ascorbic acid), 0.5mg was dried at 80 ˚C for 2 hrs. then cooled and stored over phosphorous pentoxide for 24 hrs. The weight required to prepare 50 ppm of each vitamin were dissolved in 200 ml deionized water containing 60% methanol. Diluted hydrochloric acid (0.1N) was added brought the volume to 500 ml with demonized water. Vitamins (A and E), were prepared as mentioned above.

**Results**

The current investigation provides data of the levels of vitamins (A,E and C) in serum, saliva, tumor tissue and CSF of primary brain tumor patients and normal subjects. These are well shown in tables 1, 2, 3, and 4.

Table 2 display mean concentration of vitamins in serum of primary brain tumor patients and normal subjects

### Table 2. Mean concentration of vitamins in serum of PBT patients and normal subjects.

|  |  |  |  |
| --- | --- | --- | --- |
| **Vitamin** | **Patient serum****Mean±SD****(Mg/ml)** | **Normal serum****Mean±SD****(Mg/ml)** | P value |
| **A** | **0.4436±0.2119** | **2.1±0.751** | **<0.01** |
| **C** | **3.085±1.422** | **5.66±0.691** | **<0.01** |
| **E** | **3.2166±1.363** | **4.32±0.465** | **<0.01** |

Mean concentration of vitamins in saliva of primary brain tumor patients and normal subject are shown in table 3.

### Table 3. Mean concentration of vitamins in saliva of PBT patients and normal subjects.

|  |  |  |  |
| --- | --- | --- | --- |
| **Vitamin** | **Patient saliva****Mean±SD****(Mg/ml)** | **Normal saliva****Mean±SD****(Mg/ml)** | P value |
| **A** | **0.055±0.02** | **0.227±0.14** | **<0.01** |
| **C** | **0.584±0.226** | **1.602±0.751** | **<0.01** |
| **E** | **0.396±0.142** | **0.643±0.3** | **<0.01** |

Mean concentration of vitamins in malignant and benign tissue of primary brain tumor patients are shown in table 4.

### Table 4. Mean concentration of vitamins in malignant and benign tissue of PBT patients.

|  |  |  |  |
| --- | --- | --- | --- |
| **Vitamin** | **Malignant tissue****Mean±SD****(Mg/ml)** | **Benign tissue****Mean±SD****(Mg/ml)** | P value |
| **A** | **0.667±0.191** | **0.747±0.17** | **<0.01** |
| **C** | **1.742±0.674** | **3.340±1.195** | **<0.01** |
| **E** | **1.585±0.445** | **3.081±0.725** | **<0.01** |

Table 5 shows the mean concentration of vitamins in CSF of PBT patients (both malignant and benign tumours).

### Table 5. Mean concentration of vitamins in CSF of PBT patients malignant and benign.

|  |  |  |  |
| --- | --- | --- | --- |
| **Vitamin** | **Malignant CSF****Mean±SD****(Mg/ml)** | **Benign CSF****Mean±SD****(Mg/ml)** | P value |
| **A** | **0.03±0.007** | **0.06±0.03** | **<0.01** |
| **C** | **0.52±0.035** | **1.32±0.38** | **<0.01** |
| **E** | **1.57±0.176** | **2.85±1.06** | **<0.01** |

**Vitamin A**

Serum vitamin A levels of primary brain tumor patients (0.44 ± 0.75 mg/ml) are lower than their levels in normal serum (2.1 ± 0.75 mg/ml), statistical analysis showed that this decrease is significant ( p < 0.01) as shown in table 2.

A highly significant decrease in salivary vitamin A levels of primary brain tumor patients was observed in comparison to that of normal subjects as noticed in table 3.

A significant decrease in vitamin A concentration was observed when malignant tumor tissue was compared with benign tissue, as shown in table 4.

Vitamin A levels were significantly lower in CSF of malignant PBT patients when compared with that of benign ones. The difference was significant (p < 0.01) as demonstrated in table 5.

**Vitamin E**

Table 2 demonstrates that there was a significant decrease in vitamin E serum levels of patients, when compared to its level in normal subject.

A highly significant decrease in salivary vitamin E was noticed in PBT patients in comparison to that of normal subjects, as shown in table 3.

A highly significant decrease (p < 0.01) of vitamin E levels in malignant tissue was observed in comparison to its level in benign tissue (0.58 ± 0.44 mg/ml respectively) (Table 4).

The decrease in malignant CSF content of vitamin E was highly significant in comparison to benign CSF as observed in table 5. Chromatogram of vitamin E in standard, normal serum, serum, malignant tissue and CSF of primary brain tumor patients.

**Vitamin C**

Serum vitamin C concentrations of PBT patients are lower than their levels in normal subject, as shown in table 2.

Table 3 shows a significant decrease in vitamin C level in saliva of PBT patients compared to that of normal subjects.

Vitamin C levels were significant lower in malignant tissue(1.74 ± 0.67 mg/,ml) of primary brain tumor patients when compared with that of benign tissue (3.34 ± 1.195 mg/ml) as demonstrated in table 4. Table 5 demonstrates that there was a significant decrease in vitamin C level in CSF of benign PBT.

**Discussion**

A highly significant decrease in vitamins levels (A, C, E) was observed in serum, saliva, malignant tissue and malignant CSF of PBT, as shown in the relevant tables.

These findings considered to be reasonable due to the inverse association between vitamins and cancer risks (Yochum et al., 2000, Furst and Haro, 1969, Rao et al., 2003). In addition of the fact that more ascorbic acid is used to regenerate vitamin E in membrane in these cases (Halliwell and Gutteridge, 1991). It is believed that these vitamins function as antioxidants and act as scavengers of free radicals, either independently or as part of large enzyme systems, (Nadine et.al. 1996), vitamins (A,C,E) have been postulated to play a protective role against bladder cancer (Willett W, 1990). Vitamin E is concentrated in the liquid regions that are exposed to the highest partial pressure of oxygen, such as cells lining the outer surface of the lung and red blood cell membranes. Beta-carotene is located in the membranes and organelles that are exposed to the lowest partial pressure of oxygen, but its action may not be restricted to such location, as seen by its possible protection against lung cancer (Barbara and Emily, 1996). Vitamin C is located in the water-soluble component of the body, vitamin C is believed to be the first line of defense (Frei et al. 1989, Esterbauer et al. 1992) and appears to have a role in sparing or reconstituting the active forms of vitamin E and carotenoids.

The decrease in vitamin A (retinal) levels, are characteristic of acute phase responses to infection or trauma has been known for decades (Tabone et al., Pied et al.1992). Mechanisms that have been suggested are losses of holo-retinal binding protein (holo-RBP) in the urine (Ramsdem et al., 1978, Alvarez et al., 1995), decreased release of RBP from the liver (Stephensen et al. 1994) and loss of holo-RBP into the extra cellular fluid due to increased vascular permeability (Rosales et al., 1996). However, all of these mechanisms for lowering retinal during acute phase responses postulate losses of retinal bound to RBP and ignore evidence that the molar decrease in retinal during infection or trauma frequently seen to be greater than the decreases of RBP (Thurnhamand Singkamani, 1991, Reddy et al., 1986) . Results from laboratory studies suggest that vitamin A may exhibit anticacinogenic activities that may reduce the risk of cancer, particularly, cancer of lung (Samba et al., 1990, Michaud et al. 2000) and stomach (Omenn, 1996). It has also been associated with a decreased risk of prostate cancer (Chen, 1992, Giovannucci et al., 1995).

The decrease in vitamin A concentration is due to that retinol and it analogues act as inhibitors of superoxide radical production in polymorphonuclear leukocytes (Sthelin et al., 1991). The increased risk of cancer in vitamin A deficiency is thought to be the result of a depletion in β-carotin. This compound is a very effective antioxidant and is suspected to reduce the risk of cancers known to be initiated by production of free radicals (Sthelin et al., 1991). Of particular interest is the potential benefit of increased β-carotene intake to reduce the risk of lung cancer (Rao et al., 2003).

The low levels of vitamin E concentrations might facilitate oxidative damage in

patients with brain tumor. The major site of vitamin E is to act as a natural antioxidant by scavenging free radicals and molecular oxygen (Maureen, 2004). It has other roles, unrelated to antioxidant activity, including the maintenance of cell membrane structure and effects on DNA synthesis and cell signaling (Kasai, 1997). Furthermore, vitamin E plays a crucial role in the maintenance of the immune system (Halliwell, 1997). The immune function is linked to the release of O2 radicals that participate in macrophages. Thus the immune system has been shown to be more sensitive than other systems to antioxidant deficiencies in the diet (Delafuente et al., 2000).

**Conclusions and recommendations**

These changes are quite significant, the findings should be considered in further research to determine both the specificity and sensitivity of low vitamins levels in the possible aetiological association with PBT.

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